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# Relationship Between Transfer of Drugs Through the Blood Brain Barrier and Hyperbaric Environment Concerning to C.D.P.- Choline

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原 著

Relationship Between Transfer of Drugs Through  
the Blood Brain Barrier and Hyperbaric Environment  
Concerning to C.D.P.—Choline

by

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It has been known that the transfer of pharmaceutical substances through the blood brain barrier is better in pathological conditions than in physiological conditions. For example, CDP-choline<sup>(1)</sup>, one of the central nervous drugs, is little transferred in healthy conditions<sup>(2)</sup>, but it is greater in pathologic conditions such as brain injurement<sup>(3)</sup>. In order to obtain as information concerning whether CDP-choline is transferred from blood to the brain in hyperbaric environment, we carried out the following experiments by rats. If increased transfer of

CDP-choline is observed in hyperbaric environment, it would be clinically very interesting, leading to the development of some new therapeutic approach for utilization of neuroactive drugs in the hyperbaric environment. CDP-choline metabolic pathway<sup>(4)</sup> and the chemical structure is presented in the Fig. 1, 2.

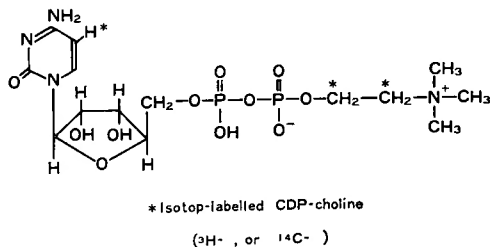


Fig. 1. Cytidine diphosphate choline

Material

Healthy Donryu rats, four weeks old, maintained under uniform conditions, were used. After the injection of  $^{14}C$ -labeled CDP-choline via the tail vein, specimens of their central nervous system tissues were taken at the intervals of the time course, and were measured for radioactivity of complex lipids, mainly phospholipid. The animals were divided into 4



made into homogenate. From the homogenate, lipids were extracted with a mixture of chloroform and methanol. The phospholipid was purified by both thin layer and alumina column chromatography and the radioactivity was determined by the scintillation counter of the ALOKA 653 type.

### Results

Results obtained from the group C in normal air environment, group B in hyperbaric oxygen environment, and in group A in hyperbaric air environment, are presented in the Fig. 4.

In the control group in normal air environment, the transfer of CDP-choline into the brain was observed, however, it was extremely small quantity. In the group B in hyperbaric oxygen environment, the transfer into the brain was found not much different from that observed in the control group. On the other hand, in the group A a significant increase in CDP-choline transfer into the brain was observed. The tendency was more marked after three hours' treatment than one hour's treatment. The natural ground radiation was  $57.7 \mu\text{Ci}$ .

In order to clarify the nature of incorporation of  $^{14}\text{C}$ -labeled CDP-choline into phospholipids of the brain tissue, the following experiments were carried out. The transfer of  $^{14}\text{C}$ -labeled CDP-choline into the brain tissue was investigated in the conventional ways<sup>(5)</sup>. The results are presented in the Fig. 5., Fig. 6.

Even in the test at the tissue level with complex factors, the transfer into the brain tended to rise in the hyperbaric groups. In the  $\text{O}_2$  hyperbaric group in this test, eight rats had been placed in a small experimental tank of 20 l capacity. These animals were taken at first under a pressure of 2 ATA, oxygen 70% and air 30%. Subsequently they were kept in the tank without ventilation.  $\text{CO}_2$  concentration in the tank was increased with time course, namely, in 60 min. 5.2%, in 180 min. 8.26%. The results are shown in the B curve of the Fig. 5.

In order to understand the above results of our present experiments, recently we had carried studies on the effect of hyperbaric environment on cerebrospinal fluid pressure under

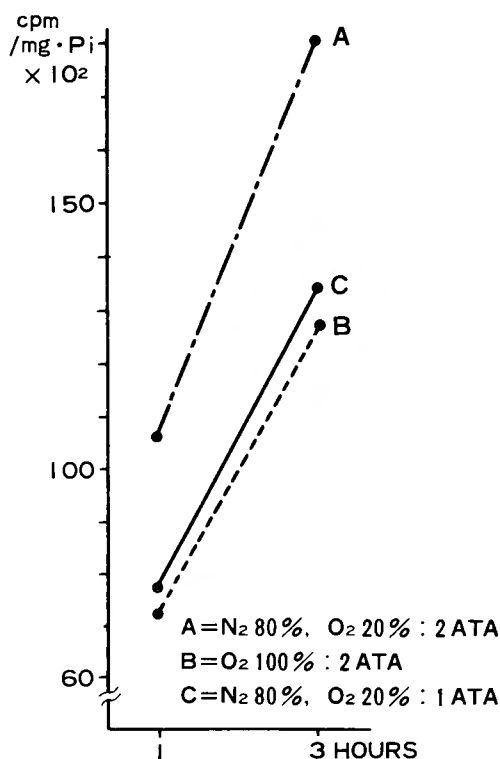


Fig. 4. Incorporation of CDP-choline-1,2- $^{14}\text{C}$  into phospholipids of the brain (rat)

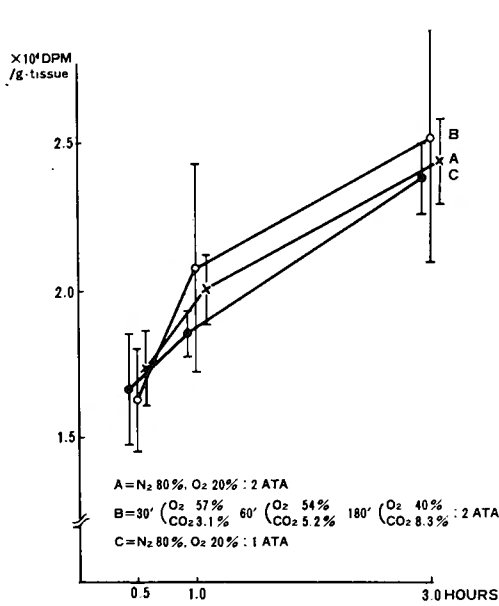


Fig. 5. Transfer of CDP-choline into brain tissue

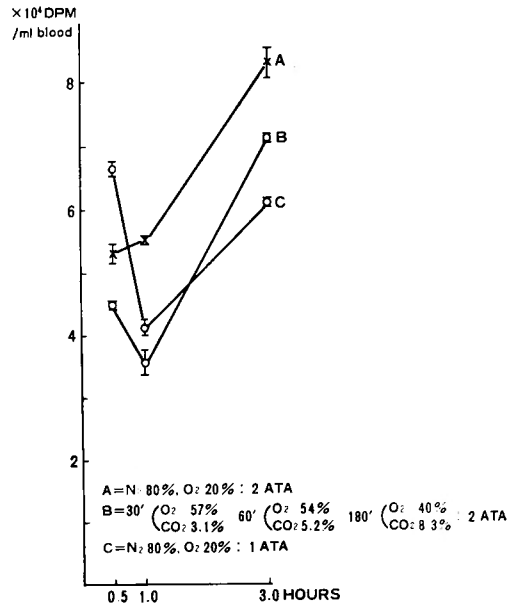


Fig. 6. Change of CDP-choline concentration in blood

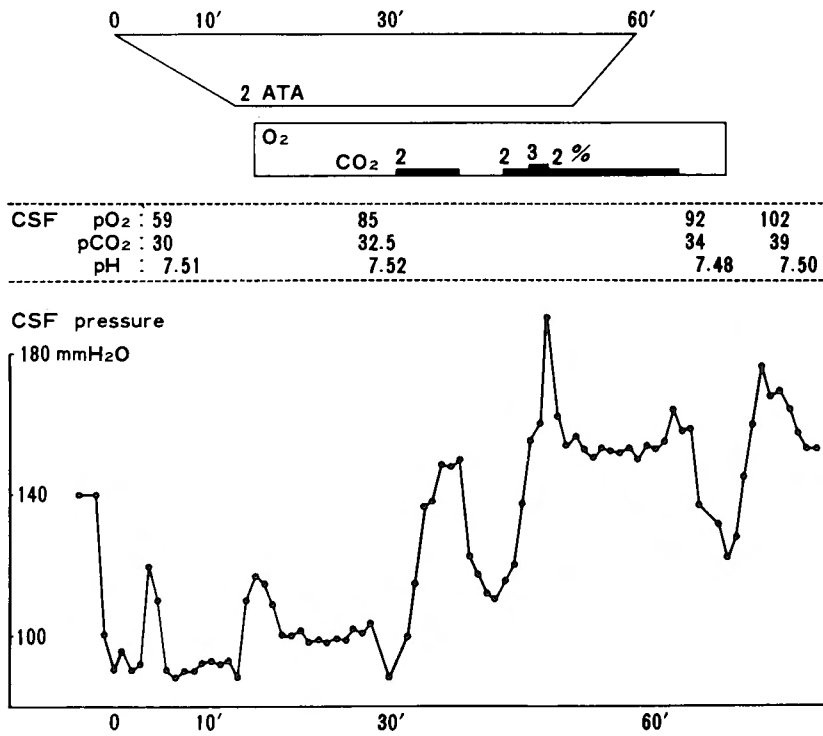


Fig. 7. Change of CSF pressure in HBO and HBO+CO<sub>2</sub>

hyperbaric oxygen incubation and CO<sub>2</sub> activation as the Fig. 7. The CSF pressure declined and the cerebral blood flow also declined, was observed under CO<sub>2</sub> activation in this study. From the above findings, it would be supported that O<sub>2</sub> or CO<sub>2</sub> concentration in the hyperbaric environment effects the function of the blood brain barrier.

To see the character of this function of the blood brain barrier, further study was undertaken with respect to the cell membrane permeability<sup>(6)</sup> of CDP-choline in the relation of the blood brain barrier. The incorporation of CDP-choline into erythrocytes, as the Fig. 8, was tested the transfer of CDP-choline into the red blood cells was not influenced.

Concentration ( $\mu$ g)	Incubation Time	$\mu$ g/ml of CDP-choline in erythrocytes
50 $\mu$ g/ml	5'	1.85
	15'	1.92
	30'	2.36
250 $\mu$ g/ml	5'	1.80
	15'	2.10
	30'	2.24
0 $\mu$ g/ml (Blank)	—	(2.07)

〈Procedure〉

Whole blood (2 ml) plus CDP-choline 100 $\mu$ g and 500 $\mu$ g.....Incubation 37°C  $\times$  5', 15' and 30'.....Centrifuge.....Erythrocytes were washed 3 times with every 2 ml physiological saline.....Hemolysis by addition of 2 ml water plus 10% TCA 2 ml.....Centrifuge.....after picking up 3 ml of top layer, wash by ether.....its water phase was cleaned up by DEAE Sephadex column.....dry by freezeipg.....dissolved into water .....Determination of High Speed Liquid Chromatography (Dyupon).

Fig. 8. Transfer of CDP-choline into erythrocytes

### Discussion

In every group, our results of the experiment showed the transfer into the brain. The tendency of its increase as the time course was noted, and it was demonstrated that CDP-choline was incorporated into lipids of brain. The fact that in the hyperbaric air environment the increased trapsfer of CDP-choline into the brain was marked in the experiment at the phospholipid level. This tendency was seen at the tissue level, too.

From these findings, it was conceivable that the increased transfer into the brain in the hyperbaric groups is due to the increase in the cerebral blood flow and the influence of CO<sub>2</sub> is also responsible, resulting vasodilation of their vasculars in the brain.<sup>(5)</sup> On the other hand, in hyperbaric O<sub>2</sub>, there is a central vasoconstriction and the cerebral blood flow decline. Therefore, it would be expected a lower value of the transfer than in the control group. However, the actual difference was not much. This may be due to the effect of hyperbaric conditions on the functon of the blood brain barrier.

### Comment

- 1) The transfer of CDP-choline which was pharmaco-active chemicals of the central nervous system, was studied, from the cerebral blood flow into the brain of the rats, through the blood brain barrier.
- 2) Resulting, some differences between the turnover rate of radioactive CDP-choline in the hyperbaric air, normal air and hyperoxic environment, were clarified.
- 3) It was assumed that this results was, due to changes of the blood brain barrier which was cussed by the means of biophysiological reactions that occurred at the hyperbaric environments and at the CO<sub>2</sub> concentration, mainly the increased cerebral blood flow, the central vascular vasodilation, and higher pressure effects.

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## 和文抄録

高気圧環境治療と薬物の脳内移行についての関係  
CDP-choline での研究

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薬物の脳内移行は、脳脊関門のため常態では移行しにくい事が知られている。我々は、中枢神経薬剤である CDP-choline をもちい、高気圧環境下、ラットでその脳内移行を調べた。ラベルした CDP-choline を尾静脈より注入し、常気圧下、高気圧下、高気圧酸素下で、径時的に脳実質を摘出し、磷脂質を中心に、比放射能を測定した。その結果、高気圧条件で移行が高

まり、高気圧空気下の方が、酸素下より、移行が高まることを知った。そこで小タンクで炭酸ガス濃度が高まる条件下では、当初の酸素下より、炭酸ガスの増加とともに、CDP-choline の脳内移行も高まる事を確かめた。この実験結果から、高気圧条件による脳脊関門の変化の他に、炭酸ガス及び酸素条件が、脳循環量に及ぼす影響も大きい事が考えられた。